

## (12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
3 July 2003 (03.07.2003)

PCT

(10) International Publication Number  
**WO 03/054228 A2**

(51) International Patent Classification<sup>7</sup>:

C12Q 1/68

(74) Agents: **GINZEL, Christian et al.**; c/o Zimmermann & Partner, Postfach 330 920, 80069 Munich (DE).

(21) International Application Number:

PCT/EP02/14578

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(22) International Filing Date:

19 December 2002 (19.12.2002)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/341,757 21 December 2001 (21.12.2001) US

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **SALASSIDIS, Konstantinos** [GR/DE]; Hauptstrasse 14c, 85386 Eching (DE). **SCHUBART, Daniel** [DE/DE]; Avenue Charles de Gaulle 1c, 13469 Berlin (DE). **GUTBROD, Heidrun** [DE/DE]; Weberhofstrasse 8, 82166 Graefelfing (DE). **MUELLER, Stefan** [DE/DE]; Thalkirchnerstrasse 184, 81371 Munich (DE). **KRAETZER, Friedrich** [DE/DE]; Geisenbrunnerstrasse 51, 81475 Munich (DE). **OBERT, Sabine** [DE/DE]; Bellinzonastrasse 17, 81475 Munich (DE).

**Published:**

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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(54) Title: HUMAN CELLULAR PROTEIN KINASES, METALLOPROTEASES AND PHOSPHATASES AS TARGETS FOR MEDICAL INTERVENTION AGAINST HEPATITIS C VIRUS INFECTIONS

WO 03/054228 A2

(57) **Abstract:** The present invention relates to human cellular protein kinases, metalloproteases and one phosphatase: beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEKS (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), and ADAM17 (U92649) as potential targets for medical intervention against Hepatitis C virus (HCV) infections. The present invention relates also to a method for the detection of compounds useful for prophylaxis and/or treatment of Hepatitis C virus infections, a method for detecting Hepatitis C virus infections in an individual or in cells. Mono- or polyclonal antibodies are disclosed effective for the treatment of HCV infections together with methods for treating Hepatitis C virus infections or for the regulation of Hepatitis C virus production and/or replication wherein said antibodies may be used. Finally the present invention relates to a solid support useful for detecting Hepatitis C virus infections or for screening compounds useful for prophylaxis and/or treatment of HCV infections.

**Human cellular protein kinases, metalloproteases and phosphatases  
as targets for medical intervention against Hepatitis C virus infections**

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**Specification**

The present invention relates to the human cellular protein kinases, metalloproteases and phosphatases:

- 10 1. beta-adrenergic receptor kinase 1 (NM\_001619, X61157, ADRBK1, GRK2, BARK1);
2. Mitogen activated protein kinase activated protein kinase 5 (AF032437, MAPKAPK5, PRAK, );
3. Insulin-stimulated protein kinase 1 (U08316, ribosomal protein S6 kinase 3 15 90K);
4. Discoidin domain receptor family, member 1 (NM\_013994, DDR1, TRK E, NEP, CAK, X74979);
5. Protein Kinase C, mu (X75756, PKC-mu);
6. Protein Kinase C, theta (L01087, PKC-theta);
- 20 7. AMP-activated protein kinase beta 2 subunit (AJ 224538, AMPK beta 2);
8. JNK2 (U09759, L31951);
9. Human p21-activated protein kinase 2 (U24153, PAK2);
10. cyclin-dependent kinase 4 (U37022, cdk4);
11. MEK5 (U25265, Mitogen-activated protein kinase kinase 5);
- 25 12. MKP-L or DUSP14, formally also named as MKP-1 like tyrosine phosphatase (NM\_007026, AF038844);
13. ADAM22 (NM\_016351, AF155382; a disintegrin and metalloproteinase domain 22);
14. ADAM17 (U92649, XM\_002270; a disintegrin and metalloproteinase domain 30 17);

as potential targets for medical intervention against Hepatitis C virus (HCV) infections. Furthermore, the present invention relates to a method for the detection of compounds useful for prophylaxis and/or treatment of Hepatitis C

virus infections and a method for detecting Hepatitis C virus infections in an individual or in cells. Also mono- or polyclonal antibodies are disclosed effective for the treatment of HCV infections together with methods for treating Hepatitis C virus infections or for the regulation of Hepatitis C virus production wherein said 5 antibodies may be used. Finally the present invention relates to a solid support useful for detecting Hepatitis C virus infections or for screening compounds useful for prophylaxis and/or treatment of HCV infections.

10 **Background of the invention**

Hepatitis C Virus (HCV) infection is a major cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma. The WHO estimates that approximately 3% of the world population, or 170 million people, have been infected with the Hepatitis C Virus. In the U.S., an estimated 3.9 million Americans have been infected (CDC 15 fact sheet Sept. 00). Over 80% of HCV-infected individuals develop chronic hepatitis, which is associated with disease states ranging from asymptomatic carrier states to repeated inflammation of the liver and serious chronic liver disease. Over the course of 20 years, more than 20% of chronic HCV-patients are expected to be at risk to develop cirrhosis or progress to hepatocellular 20 carcinoma. Liver failure from chronic hepatitis C is the leading indicator for liver transplantation. Excluding transplantation, the CDC estimates that medical and work-loss cost for HCV annually are around \$600 million. HCV is transmitted primarily by blood and blood products. Due to routine screening of the blood supplies from mid-1992, new transfusion-related cases are exceedingly rare and 25 have been surpassed by injection drug use as the highest risk factor for acquiring the virus. There is also a sexual, however inefficient, route of transmission, and a 6% rate of transmission from infected mothers to their children, which is higher in case of HIV coinfection. In a certain percentage of infections, the mode of transmission remains unknown. In spite of the significant decline in incidence in 30 the 1990's, the number of deaths (estimated deaths annually at the moment: 8000 to 10,000 in U.S.) and severe disease due to HCV is anticipated to triple in the next 10 to 20 years. (Sources: CDC fact sheet (accessed 12/12/00); Houghton M. Hepatitis C Viruses. In BN Fields, DM Knipe, PM Howley (ed.) Fields Virology.

1996. Lippencott-Raven Pub., Philadelphia; Rosen HR and Gretch DR, Molecular Medicine Today Vol5, 393, Sept. 99; Science 285, 26, July 99: News Focus: The scientific challenge of Hepatitis C; Wong JB et al, Am J Public Health, 90, 1562, Oct 2000: Estimating future hepatitis C morbidity, mortality, and costs in the  
5 United States).

According to the Consensus Statement from the EASL (European Association for the Study of the Liver) International Consensus Conference on Hepatitis C (February 26-28, 1999, Paris, France), combination therapy of alpha interferon  
10 and ribavirin is the recommended treatment for naive patients. Monotherapy with interferon has also been approved by the FDA, but the sustained response rate (HCV RNA remains undetectable in the serum for more than 6 months after end of therapy) is only 15 to 20%, in contrast to 35 to 45% with combination therapy. Interferons (Intron A, Schering-Plough; Roferon A, Hoffmann-LaRoche; Wellferon,  
15 Glaxo Wellcome; Infergen, Amgen) are injected subcutaneously three times a week, ribavirin (Rebetol, Schering-Plough) is an oral drug given twice a day. Recommended treatment duration is 6 to 12 months, depending on HCV genotype. Experimental forms of slow-release pegylated interferons (Pegasys, Hoffmann-LaRoche; PEG-Intron, Schering-Plough) have shown improvements in  
20 response rates (42 to 82% in combination with ribavirin) and application (once-weekly injection) in recent clinical studies (Hepatology32:4, Pt 2 of 2. Oct 2000; NEJM 343, 1673. Dec 00; NEJM 343, 1666. Dec 00). Common side effects of interferon therapy include: fatigue, muscle aches, head aches, nausea, fever, weight loss, irritability, depression, bone marrow suppression, reversible hair loss.  
25 The most common side effects of ribavirin are anemia, fatigue and irritability, itching, skin rash, nasal stuffiness, sinusitis, cough. More serious side effects of mono-and combination therapy occur in less than two percent of patients (NIDDK information: Chronic Hepatitis C: Current Disease Management. accessed 09.12.99). Some of the contraindications to interferon are psychosis or severe  
30 depression; neutropenia and/or thrombocytopenia; organ transplantation except liver; symptomatic heart disease; decompensated cirrhosis; uncontrolled seizures. Contraindications to ribavirin are end-stage renal failure; anemia;

hemoglobinopathies; severe heart disease; pregnancy; no reliable method of contraception (consensus statement EASL).

Experimental treatments that are not new forms of interferon are Maxamine (histamine dihydrochloride, Maxim Pharmaceuticals), which will be combined with Interferon in phase III studies, VX-497 (Vertex Pharmaceuticals), an IMP dehydrogenase inhibitor, as a less toxic ribavirin substitute in phase II, and amantadine (Endo Labs), an approved influenza drug, as the third component in triple therapy (phase II). Inhibitors for HCV enzymes such as protease inhibitors, RNA polymerase inhibitors, helicase inhibitors as well as ribozymes and antisense RNAs are under preclinical development (Boehringer Ingelheim, Ribozyne Pharmaceuticals, Vertex Pharmaceuticals, Schering-Plough, Hoffmann-LaRoche, Immusol, Merck etc.). No vaccine is available for prevention or therapeutic use, but several companies are trying to develop conventional or DNA vaccines or immunostimulatory agents (e.g. Chiron, Merck/Vical, Epimmune, NABI, Innogenetics).

In summary, the available treatment for chronic Hepatitis C is expensive, effective only in a certain percentage of patients and adverse side effects are not uncommon.

### Description of the invention

Recent research has revealed how cells communicate with each other to coordinate the growth and maintenance of the multitude of tissues within the human body. A key element of this communication network is the transmission of a signal from the exterior of a cell to its nucleus, which results in the activation or suppression of specific genes. This process is called signal transduction.

An integral part of signal transduction is the interaction of ligands, their receptors and intracellular signal transduction molecules. Ligands are messengers that bind to specific receptors on the surface of target cells. As a result of the binding, the receptors trigger the activation of a cascade of downstream signaling molecules,

thereby transmitting the message from the exterior of the cell to its nucleus. When the message reaches the nucleus, it initiates the modulation of specific genes, resulting in the production of RNA and finally proteins that carry out a specific biological function. Disturbed activity of signal transduction molecules 5 may lead to the malfunctioning of cells and disease processes. Specifically, interaction of HCV with host cells is necessary for the virus to replicate.

The present invention is based upon the discovery of a group of human cellular protein kinases, metalloproteases and one phosphatase which are specifically up- 10 or downregulated as a result of HCV replication in HCV infected host cells. The antiviral therapeutic research approach described herein focuses on discovering the cellular signal transduction pathways involved in viral infections. Identification of the signal transduction molecules, key to viral infection, provides for, among other things, novel diagnostic methods, for example, assays and compositions 15 useful therefore, novel targets for antiviral therapeutics, a novel class of antiviral therapeutics, and new screening methods (e.g. assays) and materials to discover new antiviral agents.

This approach led to the development of a novel microarray platform technology, 20 wherein a microarray of more than 900 signal transduction cDNAs was developed. This unique microarray technology was used to identify changes in RNA expression patterns (e.g. upregulation or downregulation) as a result of HCV infected host cells. Differential display techniques were used to pinpoint those signal transduction molecules useful as targets for drug intervention. Effective 25 manipulation of these virally-controlled intracellular signal transduction pathways can alter (slow or stop altogether) the course of viral growth.

It is object of the present invention to provide novel targets for medical intervention, prophylaxis and/or treatment of Hepatitis C virus infections in 30 mammals, including humans, and cells together with methods for detecting HCV infections in individuals and cells and methods for detecting compounds useful for prophylaxis and/or treatment of HCV infections. The object of the present

invention is solved by the teaching of the independent claims. Further preferred embodiments are disclosed in the dependent claims.

It is now revealed for the first time that the human cellular proteins  
5 beta-adrenergic receptor kinase 1 (gene accession number NM\_001619, other names: X61157, ADRBK1, GRK2, BARK1),  
Mitogen activated protein kinase activated protein kinase 5 (also known as: AF032437, MAPKAPK5, or PRAK),  
Insulin-stimulated protein kinase 1 (also known as: U08316, ribosomal protein S6  
10 kinase 3 90K),  
Discoidin domain receptor family, member 1 (gene accession number NM\_013994, other names: X74979, DDR1, TRK E, NEP, or CAK),  
Protein Kinase C, mu (also known as: X75756 or PKC-mu),  
Protein Kinase C, theta (also known as: L01087 or PKC-theta),  
15 AMP-activated protein kinase beta 2 subunit (also known as: AJ 224538 or AMPK beta 2),  
JNK2 (also known as: U09759 or L31951),  
Human p21-activated protein kinase 2 (also known as: U24153 or PAK2),  
cyclin-dependent kinase 4 (also known as: U37022 or cdk4),  
20 MEK5 (also known as: U25265 or Mitogen-activated protein kinase kinase 5),  
MKP-L (gene accession number: NM\_007026; also known as AF038844),  
ADAM22 (gene accession number: NM\_016351, a disintegrin and metalloproteinase domain 22, also known as AF155382),  
ADAM17 (also known as: U92649 or a disintegrin and metalloproteinase domain  
25 17 (tumor necrosis factor, alpha, converting enzyme) also known as XM\_002270),  
are specifically and uniquely up- or downregulated in a cell as a result of HCV infection. These cellular protein kinases, metalloproteases and the phosphatase therefore identify novel diagnostic and therapeutic targets for HCV infection.  
30 The only reliable experimental HCV infection studies have been performed with chimpanzees. There is no simple cell culture infection system available for HCV. Although a number of reports have been published describing *in vitro* propagation attempts of HCV in primary cells and cell lines, questions remain concerning

reproducibility, low levels of expression and properly controlled detection methods (reviewed in J. Gen Virol. 81, 1631; Antiviral Chemistry and Chemotherapy 10, 99). Thus, the replicon system described by Bartenschlager and coworkers (Lohmann et al, Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. Science 285, 110. 1999) was used for the studies disclosed herein. This replicon system reproduces a crucial part of the HCV replication cycle which is used as a system for simulating HCV infection. Bartenschlager's group produced bicistronic recombinant RNAs, so-called "replicons", which carry the Neomycin-phosphotransferase gene as well as a version of the HCV genome where the sequences for the structural HCV proteins were deleted. After transfection of the subgenomic HCV RNA molecules into the human hepatoma cell line Huh-7, cells supporting efficient RNA-dependent RNA replication of the HCV replicons were selected based on co-amplification of the neo gene and resulting resistance to the antibiotic G418. Integration of coding information into the cellular genome was an exclusion criterium for functional replicons. Several lines were established from G418 resistant clones with autonomously replicating HCV RNAs detectable by Northern blot. Minus-strand RNA replication intermediates were detected by Northern blot or metabolic radio-labeling, and the production of nonstructural HCV proteins was demonstrated by immuno-precipitation after metabolic labeling or Western blot.

Possible influences and/or dependencies of HCV's RNA-dependent RNA replication and nonstructural proteins on host cell transcription are accessible to analysis with the cDNA arrays used in the inventive methods described herein. Expression levels can be confirmed using Northern or Taqman analysis at the RNA and Western blot analysis at the protein level. Huh-pcDNA3 cells are Huh7 cells resistant to G418 by integration of a plasmid and serve as negative control. Three replicon lines were analyzed for changes in cellular RNA expression patterns compared to the control line:

- 30 ▪ Huh-9-13: cell line with persistant replicon I377/NS3-3'/wt, described in Science 1999, 285, 110-113,
- Huh-5-15: cell line with persistant replicon I389/NS3-3'/wt, described in Science 1999, 285, 110-113,
- Huh-11-7: cell line with persistant replicon I377/NS2-3'/wt, described in Science 1999, 285, 110-113.

Based on the discoveries reported herein, one aspect of the present invention is directed to a screening method for detecting compounds useful for the prophylaxis and/or treatment of Hepatitis C virus infections. Specifically, this method involves

5 contacting a test compound with at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein

10 Kinase C, mu (X75756), Protein Kinase C, theta(L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (AF038844, NM\_007026), ADAM22 (NM\_016351), ADAM17 (U92649) and detecting the human cellular protein kinase, metalloprotease or phosphatase

15 activity.

Another aspect of the present invention is directed to a diagnostic method, an assay for detecting Hepatitis C virus infections in an individual or cells. This method involves providing a sample from the individual or providing cells and detecting

20 activity of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of: beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein

25 Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), ADAM17 (U92649) and detecting the human cellular protein kinase, metalloprotease or phosphatase activity.

30

Accordingly, one aspect of the present invention is directed to novel compounds useful in the above-identified methods. Therefore, the present invention relates to a monoclonal or polyclonal antibody that binds to a human cellular protein kinase,

metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351) and ADAM17 (U92649).

10 Furthermore, the present invention discloses a method for treating Hepatitis C virus infection in an individual comprising the step of administering a pharmaceutically effective amount of an agent which inhibits at least partially the activity of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), and ADAM17 (U92649).

Another object of the present invention is to provide a method for regulating the production of Hepatitis C virus in cells comprising the step of administering a pharmaceutically effective amount of an agent to said cells wherein said agent inhibits at least partially the activity of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L

( NM\_007026), ADAM22 (NM\_016351), and ADAM17 (U92649). The above-mentioned monoclonal or polyclonal antibodies directed against these targets may be used as pharmaceutically active agents within said methods.

5 In order to identify HCV infections and new inhibitors and new pharmaceutically active compounds against Hepatitis C viruses a further aspect of the present invention is directed to a solid support useful for detecting Hepatitis C virus infections in an individual or in cells comprising an immobilized oligonucleotide, wherein said oligonucleotide is capable of detecting activity of at least one human  
10 cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated  
15 protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), and ADAM17 (U92649). Said solid support is also useful to screen compounds for the prophylaxis and/or treatment of Hepatitis C virus infections in an individual comprising at least one immobilized  
20 oligonucleotide, wherein said oligonucleotide encodes one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein  
25 Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), and ADAM17 (U92649) or comprising at least one immobilized human cellular protein kinase, metalloprotease or  
30 phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein

Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), and ADAM17 (U92649).

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Yet another aspect of the present invention is directed to a novel therapeutic composition useful for the prophylaxis and/or treatment of an individual afflicted with Hepatitis C virus comprising at least one agent capable of inhibiting activity of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), and ADAM17 (U92649).

20

#### **Detailed description of the invention**

Utilizing microarray technology, a unique microarray of more than 900 signal transduction cDNAs was developed. This array was used to compare signal transduction mRNA expression patterns (e.g. upregulation or downregulation) from HCV Replicon cells Huh-9-13, Huh-5-15, and Huh-11-7 with Huh-pcDNA control cells which do not contain HCV Replicons. These HCV Replicon cells serve as a system for simulation of HCV infected cell systems, especially for simulating HCV infected mammals, including humans. Interference of HCV with the cellular signaling events is reflected in differential gene expression when compared to cellular signaling in control cells. Results from this novel signal transduction microarray analysis revealed significant up- or downregulation of human cellular protein kinases, metalloproteases and one phosphatase. Radioactively labeled complex cDNA-probes from HCV Replicon cells Huh-9-13, Huh-5-15, and Huh-11-7 were hybridized to cDNA-arrays and compared to

hybridizations with cDNA-probes from Huh-pcDNA control cells which did not contain HCV Replicons. Surprisingly it was found that the following cellular targets are significantly up- or downregulated:

	beta-adrenergic receptor kinase 1 (NM_001619):	2.7 – 3.5 fold stronger
5	Mitogen activated protein kinase activated protein kinase 5 (AF032437):	2.2 – 3.0 fold stronger
	Insulin-stimulated protein kinase 1 (U08316):	2.2 – 3.1 fold stronger
	TRK E (NM_013994):	3.2 -10.3 fold stronger
	Human p21-activated protein kinase 2 (U24153):	1.8 – 2.7 fold stronger
10	PKC-mu (X75756):	2.3 – 3.2 fold weaker
	PKC-theta (L01087):	2.6 – 3.3 fold weaker
	AMP-activated protein kinase beta 2 subunit (AJ 224538):	1.9 – 2.2 fold weaker
	JNK2 (U09759):	2.6 – 4.1 fold weaker
15	cdk4 (U37022)	1.8 – 3.3 fold stronger
	MEK5 (U25265)	0.9 – 3.6 fold stronger
	MKP-L (NM_007026):	2.1 – 2.3 fold weaker
	ADAM22 (NM_016351):	2.5 – 3.8 fold weaker
20	ADAM17 (U92649):	3.4 – 3.8 fold weaker

Disclosed herein is the first report describing the role of human cellular proteins beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein

kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), ADAM17 (U92649) in the signal transduction of the HCV infection process. As a result of these discoveries, novel compounds and inhibitors against the above-mentioned human cellular protein kinases, metalloproteases and phosphatases may be found by the use of the inventive methods disclosed herein.

ADAM17 and ADAM22 are proteins of the ADAM family (proteins containing a disintegrin and metalloprotease domain). ADAM17 is also known as "Homo sapiens a disintegrin and metalloproteinase domain 17" and ADAM22 is known as "Homo sapiens metalloprotease-like, disintegrin-like, cysteine-rich protein 2 epsilon".

As used herein, the term "inhibitor" refers to any compound capable of downregulating, decreasing, suppressing or otherwise regulating the amount and/or activity of the human cellular proteins beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), and ADAM17 (U92649). Generally, human cellular protein inhibitors may be proteins, oligo- and polypeptides, nucleic acids, small chemical molecules, or other chemical moieties.

As used herein, the term "regulating expression and/or activity" generally refers to any process that functions to control or modulate the quantity or activity (functionality) of a cellular component. Static regulation maintains expression and/or activity at some given level. Upregulation refers to a relative increase in expression and/or activity. Accordingly downregulation refers to a relative decrease in expression and/or activity. In the present invention, regulation is preferably downregulation of a cellular component. As used herein,

downregulation is synonymous with inhibition of a given cellular component's activity.

Therapeutics, pharmaceutically active agents or inhibitors, respectively, may be  
5 administered to cells from an individual *in vitro*, or may involve *in vivo*  
administration to the individual. The term "individual" preferably refers to  
mammals and most preferably to humans. Routes of administration of  
pharmaceutical preparations to an individual may include oral and parenteral,  
including dermal, intradermal, intragastral, intracutan, intravasal, intravenous,  
10 intramuscular, intraperitoneal, intranasal, intravaginal, intrabuccal, percutan,  
rectal, subcutaneous, sublingual, topical or transdermal application, but are not  
limited to these ways of administration. For instance, the preferred preparations  
are in administratable form which is suitable for oral application. These  
15 administratable forms, for example, include pills, tablets, film tablets, coated  
tablets, capsules, powders and deposits. Administration to an individual may be in  
a single dose or in repeated administrations, and may be in any of a variety of  
physiologically acceptable salt forms, and/or with an acceptable pharmaceutical  
carrier, binder, lubricant, excipient, diluent and/or adjuvant. Pharmaceutically  
acceptable salt forms and standard pharmaceutical formulation techniques are  
20 well known to persons skilled in the art.

As used herein, a "pharmaceutical effective amount" of a human cellular protein  
kinase, metalloprotease or phosphatase inhibitor is an amount effective to achieve  
the desired physiological result, either in cells treated *in vitro* or in a subject  
25 treated *in vivo*. Specifically, a pharmaceutically effective amount is an amount  
sufficient to inhibit, for some period of time, one or more of the clinically defined  
pathological processes associated with the viral infection. The effective amount  
may vary depending on the specific human cellular protein kinase,  
metalloprotease or phosphatase inhibitor selected, and is also dependent on a  
30 variety of factors and conditions related to the subject to be treated and the  
severity of the infection. For example, if the inhibitor is to be administered *in vivo*,  
factors such as the age, weight and health of the patient as well as dose response  
curves and toxicity data obtained in pre-clinical animal work would be among

those considered. If the inhibitor is to be contacted with the cells *in vitro*, one would also design a variety of pre-clinical *in vitro* studies to assess such parameters as uptake, half-life, dose, toxicity, etc. The determination of a pharmaceutically effective amount for a given agent is well within the ability of  
5 those skilled in the art.

It is also apparent to a person skilled in the art that detection includes any method known in the art useful to indicate the presence, absence, or amount of a detection target. Such methods may include, but are not limited to, any molecular  
10 or cellular techniques, used singularly or in combination, including, but not limited to: hybridization and/or binding techniques, including blotting techniques and immunoassays; labeling techniques (chemiluminescent, colorimetric, fluorescent, radioisotopic); spectroscopic techniques; separations technology, including precipitations, electrophoresis, chromatography, centrifugation, ultrafiltration, cell  
15 sorting; and enzymatic manipulations (e.g., digestion).

The present disclosure teaches for the first time the up- or downregulation of a group of human cellular protein kinases, metalloproteases and a phosphatase specifically involved in the viral infection of Hepatitis C virus. Thus, the present  
20 invention is also directed to a method useful for detecting novel compounds useful for prophylaxis and/or treatment of HCV infections.

Methods of the present invention identify compounds useful for prophylaxis and/or treatment of HCV infections by screening a test compound, or a library of test  
25 compounds, for its ability to inhibit any one or more of the group of human cellular protein kinases, metalloproteases or phosphatases identified herein as characteristically up- or downregulated during HCV growth and RNA replication inside a cell. A variety of assay protocols and detection techniques are well known in the art and easily adapted for this purpose by a skilled practitioner. Such  
30 methods include, but are not limited to, high throughput assays (e.g., microarray technology, phage display technology), and *in vitro* and *in vivo* cellular and tissue assays.

In a related aspect, the present invention provides, in view of the discovery of human cellular protein kinases, metalloproteases and phosphatases specifically involved in the HCV infection process, an assay component specially useful for detecting HCV in an individual or in cells. Preferably the assay component

5 comprises oligonucleotides immobilized on a solid support capable of detecting activity of one or more of the human cellular protein kinases, metalloproteases or phosphatase comprising:

the kinases beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5

10 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1

(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265);

15 the metalloproteases ADAM22 (NM\_016351), ADAM17 (U92649) and

the phosphatase MKP-L (NM\_007026).

20 Preferably the solid support would contain oligonucleotides of sufficient quality and quantity to detect all of the above-mentioned human cellular proteins (e.g., a nucleic acid microarray).

Similarly, it is an object of the present invention to provide an assay component  
25 specially useful for screening compounds useful for the prophylaxis and/or treatment of HCV infections. One preferred assay component comprises oligonucleotides that encode one or more human cellular protein kinases: beta-adrenergic receptor kinase (X61157), Mitogen activated protein kinase activated protein kinase (AF032437), Insulin-stimulated protein kinase 1 (U08316), TRK E (X74979), PKC-mu (X75756), PKC-theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (L31951), Human p21-activated protein kinase, and PAK2 (U24153); or metalloproteases: ADAM22 (AF155382) and ADAM17

(XM\_002270); or the Phosphatase MKP-L, (NM\_007026) immobilized on a solid support.

In another embodiment, the assay component comprises peptide fragments of  
5 one or more of the above-identified human cellular proteins immobilized on a solid support. Once again the most preferred solid support embodiment would contain polymers of sufficient quality and quantity to detect all of the above-mentioned human cellular protein kinases, metalloproteases and the phosphatase (e.g., a nucleic acid or a peptide microarray). A variety of supports and constructions of  
10 the same for the methods disclosed herein are well known in the art and easily adapted for this purpose by a skilled practitioner (cf., for example: Marschall, 1999 "Do-it-yourself gene watching" Science 286, 444-447; Service 2000 "Protein arrays step out of DNA's shadow" Science 289, 1673).

15 It is preferred that mRNA is measured as an indication of expression. Methods for assaying for mRNA include, but are not limited to, Northern blots, slot blots, dot blots, and hybridization to an ordered array of oligonucleotides. Nucleic acid probes useful for assay of a sample are preferably of sufficient length to specifically hybridize only to appropriate, complementary transcripts. Typically the  
20 oligonucleotide probes will be at least 10 to 25 nucleotides in length. In some cases longer probes of at least 30, 40, or 50 up to 2500 nucleotides will be desirable.

The polypeptide product of gene expression may be assayed to determine the  
25 amount of expression as well. Methods for assaying for a protein include, but are not limited to, western blot, immuno-precipitation, radioimmuno assay, and peptide immobilization in an ordered array. It is understood, however, that any method for specifically and quantitatively measuring a specific protein or mRNA product can be used.

30

A variety of supports upon which nucleic acids or peptides can be immobilized are known in the art, for example filters, or polyvinyl chloride dishes. Any solid surface to which oligonucleotides or peptides can be bound, either directly or indirectly,

either covalently or non-covalently, can be used. A preferred solid support is a microarray membrane filter or a "biochip". These contain particular polymer probes in predetermined locations on the array. Each predetermined location may contain more than one molecule of the probe, but each molecule within the 5 predetermined location has an identical sequence.

The present invention incorporates by reference in their entirety techniques well known in the field of molecular biology. These techniques include, but are not limited to, techniques described in the following publications:

10 Ausubel, F.M. et al. eds., "Short Protocols In Molecular Biology" 4<sup>th</sup> Ed. 1999, John Wiley & Sons, NY (ISBN 0-471-32938-X);  
Old, R.W. & S.B. Primrose "Principles of Gene Manipulation: An Introduction To Genetic Engineering" 3<sup>rd</sup> Ed. 1985, Blackwell Scientific Publications, Boston.  
Studies in Microbiology: V.2, 409 pp. (ISBN 0-632-01318-4);

15 Miller, J.H. & M.P. Calos eds., "Gene Transfer Vectors For Mammalian Cells" 1987, Cold Spring Harbor Laboratory Press, NY. 169 pp. (ISBN 0-87969-198-0);  
Mayer, R.J. & J.H. Walker eds. "Immunochemical Methods In Cell and Molecular Biology" 1987, Academic Press, London. 325 pp. (ISBN 0-12480-855-7);  
Sambrook, J. et al. eds., "Molecular Cloning: A Laboratory Manual" 2<sup>nd</sup> Ed. 1989,  
20 Cold Spring Harbor Laboratory Press, NY. Vols. 1-3. (ISBN 0-87969-309-6);  
Winnacker, E.L. "From Genes To Clones: Introduction To Gene Technology" 1987  
VCH Publishers, NY. (translated by Horst Ibelgauf) 634 pp. (ISBN 0-89573-614-4).

25 The present invention further incorporates by reference in their entirety techniques well known in the field of microarray construction and analysis. These techniques include, but are not limited to, techniques described in the following patents and patent applications describing array of biopolymeric compounds and methods for their fabrication:

30 U.S. Pat. Nos. 5,242,974; 5,384,261; 5,405,783; 5,412,087; 5,424,186;  
5,429,807; 5,436,327; 5,445,934; 5,472,672; 5,527,681; 5,529,756;  
5,545,531; 5,554,501; 5,556,752; 5,561,071; 5,559,895; 5,624,711;

5,639,603; 5,658,734; 5,807,522; 6,087,102; WO 93/17126; WO  
95/11995; WO 95/35505; EP 742 287; and EP 799 897.

Techniques also include, but are not limited to, techniques described in the following  
5 patents and patent application describing methods of using arrays in various  
applications:

U.S. Pat. Nos. 5,143,854; 5,288,644; 5,324,633; 5,432,049;  
5,470,710; 5,492,806; 5,503,980; 5,510,270; 5,525,464; 5,547,839;  
5,580,732; 5,661,028; 5,994,076; 6,033,860; 6,040,138; 6,040,140;  
10 WO 95/21265; WO 96/31622; WO 97/10365; WO 97/27317;  
EP 373 203; and EP 785 280

It is readily apparent to those skilled in the art that other suitable modifications and  
adaptations of the compositions and methods of the invention described herein are  
15 evident and may be made without departing from the scope of the invention or the  
embodiments disclosed herein. Having now described the present invention in  
detail, the same will be more clearly understood by reference to the following  
examples, which are included for purposes of illustration only and are not intended  
to be limiting of the invention.

20

## Examples

### Materials and Methods

25

#### 1. Generation of cDNA-arrays on membranes

In order to manufacture cDNAs-arrays on membranes, the following strategy was  
pursued: cDNAs encoding parts of or full length proteins of interest – in the  
following referred to as “target cDNAs” – were cloned into the plasmid Bluescript II  
30 KS<sup>+</sup> (Stratagene, USA). Large scale purifications of these plasmids were  
performed according to standard techniques and 200 µl aliquots (1 µg/µl plasmid  
concentration) were transferred into appropriate 96 well plates. Plates were  
closed with sealing tape and chilled on ice for 5 minutes after incubation for 10  
minutes at 95°C. 10 µl of 0.6N NaOH were added and the mix was stored for 20

minutes at room temperature before addition of 10 µl 2.5M Tris-HCl pH 7.1 and 20 µl 40x SSC. Target cDNAs were spotted onto Nylon or Nitrocellulose membranes using a BioGrid (BioRobotics, UK) equipped with a 0.7 mm pintoil. In this way, between 200 ng and 350 ng of plasmids encoding target cDNAs were transferred 5 onto the membranes and crosslinked to the membranes by ultraviolet light ( $1.2 \times 10^5 \mu\text{J}/\text{cm}^2$ ). The arrays were stored for use in subsequent experiments at at 4°C.

## 2. Cellular HCV RNA replication system

10 Huh-pcDNA3, Huh-9-13, Huh-5-15 and Huh-11-7 cells were grown in DMEM supplemented with 10% FCS, 2 mM Glutamine, Penicillin (100 IU/ml) / Streptomycin (100 µg/ml) and 1x nonessential amino acids in the presence of 1 mg/ml G418. Cells were routinely passaged three times a week at a dilution of 1:3 or 1:2.

15

## 3. Lysis of cells, and isolation of total RNA

Huh-pcDNA3, Huh-9-13, Huh-5-15 and Huh-11-7 cells were seeded at  $5 \times 10^5$  cells per 10 cm plate in medium without G148. The medium was changed 3 days after plating and cells were harvested 5 days after plating by lysing the cells 20 directly on the plate with 4 ml of Tri reagent (Molecular Research Center, Inc., USA). The lysates were stored at room temperature for 5 minutes and then centrifuged at 12000xg for 15 minutes at 4°C. The supernatant was mixed with 0,1 ml of 1-Bromo-3-chloropropane per 1 ml of Tri reagent and vigorously shaken. The suspension was stored for 5 minutes at room temperature and then 25 centrifuged at 12000xg for 15 minutes at 4°C. The colorless upper phase was transferred into new tubes, mixed with 5 µl of polyacryl-carrier (Molecular Research Center Inc., USA) and with 0.5 ml of isopropanol per 1 ml of Tri reagent and vigorously shaken. The samples were stored at room temperature for 5 minutes and then centrifuged at 12000xg for 8 minutes at 4°C. The supernatant 30 was removed and the RNA pellet washed twice with 1 ml of 75% Ethanol. The pellet was dried and resuspended in 25 µl of RNase-free buffer per initial 1 ml lysate.

#### 4. Preparation of radioactively labelled cDNA probes from RNA

In order to obtain a radioactively labeled cDNA probe, RNA was transcribed into a cDNA-probe in the presence of radioactively labeled dATP. 12 µl bidestilled DEPC treated H<sub>2</sub>O containing 1 µg of primer TXN (5'-TTT TTT TTT TTT TTT TXN-3' with T → dTTP; N → dATP, dCTP, dGTP or dTTP; X → dATP, dCTP or dGTP) and total RNA (6 µg) were shaken between 5 and 15' at 60°C and then 5 incubated at 4°C for 2 to 10 minutes. After centrifugation (30 seconds, 10000xg) 7 µl of a mix consisting of 100 µCi dATP-P<sup>33</sup> (Amersham, UK) which were dried under vacuum previously and resuspended in 4 µl first strand buffer (Life 10 Technologies, USA), 2 µl 0.1M DTT and 1 µl labeling solution (4mM dCTP, dGTP, dTTP each and 80 µM dATP final concentration) were added. Following the addition of 1 µl Superscript II reverse transcriptase (Life Technologies, USA) the reaction was incubated for 10 minutes at room temperature and then for 60 minutes at 38°C. Subsequently, the reaction was vigorously shaken for 30 15 minutes at 68°C after adding 5 µl 0.5M EDTA and 25 µl 0.6M NaOH.

Unincorporated nucleotides were removed from the labeling reaction using ProbeQuant G-50 columns (Amersham, UK). The column was vigorously shaken and centrifuged for 1 minute at 735xg in an appropriate reaction tube after bottom 20 closure and lid were removed. The column was placed into a new reaction tube, the probe was applied onto the center of the column material and the column was centrifuged for 2 minutes at 735xg. The flow-trough was transferred into new reaction tubes and filled up to a volume of 100 µl with 10mM Tris, pH 7.4, 1 mM EDTA. The probe was precipitated by centrifugation for 15 minutes at 12000xg 25 after 4 µl of 5M NaCl, 1 µl polyacryl-carrier (Molecular Research Center Inc., USA) and 250µl Ethanol were added. The supernatant was discarded and the pellet dried before starting with the hybridisation.

30

#### 5. Hybridisation of radioactively labeled cDNA-probes to cDNA-arrays

The pellet was resuspended in 10 µl C<sub>0</sub>T DNA (1 µg/µl, Roche Diagnostics, Germany), 10 µl yeast tRNA (1 µg/µl Sigma, USA) and 10 µl polyA (1 µg/µl, Roche

Diagnostics, Germany). Herring sperm DNA was added to a final concentration of 100 µg/ml and the volume was filled up to 100 µl with 5 µl 10% SDS (Sodiumdodecylsulfat), 25 µl 20x SSPE and bidestilled H<sub>2</sub>O. The mix was put on 95°C for 5 minutes, centrifuged for 30 seconds at 10000xg and vigorously shaken  
5 for 60 minutes at 65°C. A 1 µl aliquot of the probe was used to measure the incorporation of radioactive dATP with a scintillation counter. Probes with at least a total of 20x10<sup>6</sup> cpm were used. The arrays were prehybridised for at least 3 hours at 65°C in hybridisation solution in a roller bottle oven. After  
10 prehybridisation the radioactively labeled probe was added into the hybridisation solution and hybridisation was continued for 20 hours. The probe was discarded and replaced with wash solution A (2xSSC). The arrays were washed twice in wash solution A at room temperature in the roller oven. Afterwards, wash solution A was replaced by wash solution B (2x SSC, 0.5% SDS) preheated to 65°C and arrays were washed twice for 30 minutes at 65°C. Then, wash solution B was  
15 replaced by wash solution C (0.5x SSC, 0.5% SDS) preheated to 65°C and arrays were washed twice for 30 minutes at 65°C. The moist arrays were wrapped in airtight bags and exposed for 8 to 72 hours on erased phosphoimager screens (Fujifilm, Japan).

20 **6. Analysis of cDNA-arrays**

The exposed phosphoimager screens were scanned with a resolution of 100 µ and 16bits per pixel using a BAS-1800 (Fujifilm, Japan). Files were imported into the computer program ArrayVision (Imaging Research, Canada). Using the program's features, the hybridisation signals of each target cDNA were converted  
25 into numbers. The strength of the hybridisation signals reflected the quantity of RNA molecules present in the probe. Differentially expressed genes were selected according to the ratio of their signal strength after normalization to the overall intensity of the arrays.

30

**7. Analysis of expression levels by Northern blot experiment**

Huh-pcDNA3, Huh-9-13, Huh-5-15 and Huh-11-7 cells were seeded at 5 x 10<sup>5</sup> cells per 10 cm plate in medium without G148. Cells were harvested after 3 days

by lysing the cells directly on the plate with 4 ml of Tri reagent (Molecular Research Centre, Inc., USA). The lysates were stored at room temperature for 5 minutes and then centrifuged at 12000xg for 15 minutes at 4°C. The supernatant was mixed with 0,1 ml of 1-bromo-3-chloropropane per 1 ml of Tri reagent and 5 vigorously shaken. The suspension was stored for 5 minutes at room temperature and then centrifuged at 12000xg for 15 minutes at 4°C. The colourless upper phase was transferred into new tubes, mixed with 5 µl of poly-acryl-carrier (Molecular Research Centre, Inc., USA) and with 0.5 ml of isopropanol per 1 ml of Tri reagent and vigorously shaken. The samples were stored at room temperature 10 for 5 minutes and then centrifuged at 12000xg for 8 minutes at 4°C. The supernatant was removed and the RNA pellet washed twice with 1 ml of 75% Ethanol. The pellet was dried and resuspended in 25 µl of RNase-free water per initial 1 ml lysate. 8 µg of total RNA per sample was loaded onto formaldehyde-containing agarose gels (Sambrook et al. Cloning manual, CSHL press, 1989) and 15 transferred to HYbond NX membranes (Amersham) overnight in 20x SSC (3M NaCl, 300mM C<sub>6</sub>H<sub>5</sub>Na<sub>3</sub>O<sub>7</sub> x 2 H<sub>2</sub>O, pH 7.0) by capillary transfer. RNA was immobilized to the filter using UV-crosslinking (120mJ/cm<sup>2</sup> for 25 seconds). Filters were hybridized to oligonucleotide probes or random-primed probes specific for the genes in question. Quantitation of signals was performed with a Fuji 20 phosphoimager.

#### 8. Verification of de-regulated genes by quantitative Real-Time PCR

Quantitative RT-PCR was used to verify hits resulting from DNA macroarray experiments by exploiting the 5'-exonuclease of Taq DNA polymerases to cleave 25 the 5' fluorescent label of an oligonucleotide. Total RNA was extracted from cell lines (Qiagen RNeasy Mini Kit, QIAGEN, Hilden) and was reverse transcribed with Superscript II (Invitrogen, Karlsruhe) according to the manufacturer's protocol with 5 µg of RNA as a template and oligodT primers. Subsequently, the cDNA was analysed on a ABI PRISM 7000 Sequence Detection System (Applied 30 Biosystems, Darmstadt) with the 5'exonuclease assay by using the TaqMan Universal PCR Master Mix (#4324018, Applied Biosystems, Darmstadt) and non-extendible oligonucleotides. Gene-specific TaqMan probes were labelled with the reporter dye FAM™ at the 5'-end and the quencher dye TAMRA™ at the 3' end of

the probe. GAPDH and 18SrRNA were used as reference genes with TaqMan probes that were labelled with VIC<sup>TM</sup> and TAMRA<sup>TM</sup> accordingly.

Experimental conditions were 2 minutes 50°C, 10 minutes 95°C, followed by 40 cycles with 15 seconds at 95°C and 1 minute at 60°C. Primer Express software

5 was used to design primers with a melting temperature of 58 - 60°C amplifying an amplicon of a maximum length of 150 bp.

#### 9. Analysis of expression levels by Western blot experiments

Huh-pcDNA3, Huh-9-13, Huh-5-15 and Huh-11-7 cells were seeded at 5 x 10<sup>5</sup>

10 cells per 10 cm plate in medium without G148. Cells were harvested after 3 days by the addition of 500 µl of 1x SDS sample buffer (62.5 mM Tris-HCl pH 6.8, 2% w/v SDS, 10% glycerol, 50 mM DTT, 0.01% bromphenol blue) or RIPA lysis buffer. Lysates were separated on SDS-poly acrylamide gels and proteins transferred to nitrocellulose. Western blotting was performed with the appropriate  
15 antibodies according to the manufacturers instructions.

**Claims**

1. A method for detecting compounds useful for the prophylaxis and/or treatment of Hepatitis C virus infections, the method comprising the following steps:
  - a) contacting a test compound with at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), ADAM17 (U92649); and
  - b) determining the activity of said human cellular protein kinase, metalloprotease or phosphatase.
2. A method for detecting Hepatitis C virus infections in an individual, the method comprising the following steps:
  - a) providing a sample from said individual; and
  - b) determining the activity in said sample of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4

(U37022), MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), and ADAM17 (U92649).

3. A method for detecting Hepatitis C virus infections in cells, the method  
5 comprising the following steps:

a) providing said cells; and  
b) determining the activity in said cells of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), and ADAM17 (U92649).

4. An antibody that binds to a human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), and ADAM17 (U92649).

5. The antibody of claim 4, wherein said antibody is a monoclonal or polyclonal antibody.

30  
6. A method for treating Hepatitis C virus infection in an individual, the method comprising the step of administering a pharmaceutically effective amount of an

agent which inhibits at least partially the activity of at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026),  
10 ADAM22 (NM\_016351), and ADAM17 (U92649).

7. A method for regulating the production of Hepatitis C virus in cells, the method comprising the step of administering a pharmaceutically effective amount of an agent to said cells wherein said agent inhibits at least partially the activity of at  
15 least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026),  
20 ADAM22 (NM\_016351), and ADAM17 (U92649).
- 25 8. The method according to claim 6 or 7, wherein the agent is an antibody which binds to at least one human cellular protein kinase, metalloprotease or phosphatase selected from the group consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein  
30

kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), and ADAM17 (U92649).

9. The method according to claim 8, wherein said antibody is a monoclonal or  
5 polyclonal antibody.
10. A solid support useful for detecting Hepatitis C virus infections in an individual,  
the solid support comprising an immobilized oligonucleotide, wherein said  
oligonucleotide is capable of detecting activity of at least one human cellular  
protein kinase, metalloprotease or phosphatase selected from the group  
10 consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen  
activated protein kinase activated protein kinase 5 (AF032437), Insulin-  
stimulated protein kinase 1 (U08316), Discoidin domain receptor family,  
member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C,  
15 theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538),  
JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-  
dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026),  
ADAM22 (NM\_016351), and ADAM17 (U92649).
- 20 11. A solid support useful for detecting Hepatitis C virus infections in cells, the  
solid support comprising an immobilized oligonucleotide, wherein said  
oligonucleotide is capable of detecting activity of at least one human cellular  
protein kinase, metalloprotease or phosphatase selected from the group  
consisting of beta-adrenergic receptor kinase 1 (NM\_001619), Mitogen  
activated protein kinase activated protein kinase 5 (AF032437), Insulin-  
25 stimulated protein kinase 1 (U08316), Discoidin domain receptor family,  
member 1(NM\_013994), Protein Kinase C, mu (X75756), Protein Kinase C,  
theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538),  
JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-  
30 dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026),  
ADAM22 (NM\_016351), and ADAM17 (U92649).

12. A solid support useful for screening compounds useful for the prophylaxis  
and/or treatment of Hepatitis C virus infections in an individual, the solid  
support comprising at least one immobilized oligonucleotide, wherein said  
oligonucleotide encodes one human cellular protein kinase, metalloprotease or  
phosphatase selected from the group consisting of beta-adrenergic receptor  
5 kinase 1 (NM\_001619), Mitogen activated protein kinase activated protein  
kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin  
domain receptor family, member 1(NM\_013994), Protein Kinase C, mu  
(X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase  
10 beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein  
kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265),  
MKP-L (NM\_007026), ADAM22 (NM\_016351), and ADAM17 (U92649).

13. A solid support useful for screening compounds useful for the prophylaxis  
15 and/or treatment of Hepatitis C virus infections in an individual, the solid  
support comprising at least one immobilized human cellular protein kinase,  
metalloprotease or phosphatase selected from the group consisting of beta-  
adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase  
activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1  
20 (U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein  
Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated  
protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-  
activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022),  
MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), and  
25 ADAM17 (U92649).

14. A composition useful for the prophylaxis and/or treatment of an individual  
afflicted with Hepatitis C virus, the composition comprising at least one agent  
capable of inhibiting activity of at least one human cellular protein kinase,  
30 metalloprotease or phosphatase selected from the group consisting of beta-  
adrenergic receptor kinase 1 (NM\_001619), Mitogen activated protein kinase  
activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1  
(U08316), Discoidin domain receptor family, member 1(NM\_013994), Protein

Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEK5 (U25265), MKP-L (NM\_007026), ADAM22 (NM\_016351), and  
5 ADAM17 (U92649).

**10. Nucleic acid and amino acid sequences of human cellular proteins:**

DEFINITION Homo sapiens adrenergic, beta, receptor kinase 1 (ADRBK1), mRNA.

5 ACCESSION NM\_001619  
EC number = "2.7.1.126"

10 DEFINITION Homo sapiens mitogen activated protein kinase activated protein kinase gene, complete cds.

15 ACCESSION AF032437  
VERSION AF032437.1 GI:3133290  
FEATURES Location/Qualifiers  
source 1..1968  
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/db\_xref="taxon:9606"

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LDAPVKLCDGFRAKIDQGDLMTQPQFTPYYVAPQVLEAQRRHQKEKSGIIPSTSPTPYTY

35 NKSCDLWSLGVIYVMLCGYPPFYSKHHSRTIPKDMRRKIMTGSFEFPEEEWSQISEM  
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40 EQLANMRIQDLKVSLSKPLHSVNNPILRKRKLLGTPKDKDSVYIHDHENGAEDSNVALEK  
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45 BASE COUNT 571 a 445 c 485 g 467 t  
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15

20 DEFINITION Human insulin-stimulated protein kinase 1 (ISPK-1) mRNA,  
 complete  
 cds.  
 ACCESSION U08316  
 VERSION U08316.1 GI:475587  
 25 FEATURES Location/Qualifiers  
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BASE COUNT 704 a 417 c 510 g 629 t

ORIGIN

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 181 gaaaaggcag atccctccca gtttgaactt taaaaggat tagggcaggg atcatttgg  
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50

DEFINITION Homo sapiens discoidin domain receptor family, member 1 (DDR1), transcript variant 3, mRNA.

ACCESSION NM\_013994

VERSION NM\_013994.1 GI:7669484

55 EC number = "2.7.1.112"

60

DEFINITION H.sapiens mRNA for protein kinase C mu.

ACCESSION X75756

VERSION X75756.1 GI:438372

FEATURES Location/Qualifiers

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DEFINITION Human protein kinase C-theta (PRKCT) mRNA, complete cds.  
 50 ACCESSION L01087  
 VERSION L01087.1 GI:558098  
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	481	ggacatgaat	gaatttgaga	cggaaggctt	ctttgcittg	catcagcgcc	gggggtccat
	541	caagcaggca	aagggtccacc	acgtcaagtgc	ccacgagttc	actgcccac	tcttccacaca
	601	gcccacattt	tgctctgtct	gccacgagtt	tgtctggggc	ctgaacaaac	agggttacca
	661	gtgcccacaa	tgcaatgcag	caattcaca	gaagtgtatt	gataaaagtta	tagcaaagt
	721	cacaggatca	gctatcaata	gccgagaaaac	catgttccac	aaggagagat	tcaaaattga
	781	catgccacac	agatttaaaag	tctacaatta	caagagcccg	accttctgtg	aacactgtgg
	841	gaccctgctg	tggggactgg	cacggcaagg	actcaagtgt	gatgcatgt	gcatgaatgt
	901	gcatcataga	tgccagacaa	aggtggccaa	cctttgtggc	ataaaaccaga	agctaataggc
	961	tgaagcgctg	gccatgattt	agagactca	acaggctcgc	tgtttaagag	atactgaaca
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	1081	gctgccatgt	ttaccgcac	cgggaaaaaaag	agagcctcag	ggcatttcct	gggagtctcc
	1141	gttggatgag	gtggataaaa	tgtgccatct	tccagaacct	gaactgaaca	aagaaagacc
	1201	atctctgcag	attaaactaa	aaattgagga	ttttatcttgc	cacaaaatgt	tggggaaagg
	1261	aagttttggc	aaggcttcc	tggcagaatt	caagaaaaacc	aatcaatttt	tcgcaataaa
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	1381	gagagttctt	tccttggcct	gggagcatcc	gtttctgacg	cacatgtttt	gtacatttca
	1441	gaccaaggaa	aaccttcttt	ttgtgcatttgc	gtacctaacc	ggaggggact	taatgtacca
	1501	catccaaagc	tgccacaagt	tcgaccccttc	cagagcgacg	ttttatgtcg	ctgaaatcat
	1561	tcttggctcg	cagttcccttc	attccaaagg	aatagtctac	aggacactga	agctagataa
	1621	catccgttta	gacaaagatg	gacatataaa	gatcgccgat	tttggaaatgt	gcaaggagaa
	1681	catgttagga	gtgcacaa	cgaataccctt	ctgtggacca	cctgactaca	tcgccccaga
	1741	gatcttgctg	ggtcagaat	acaaccactc	tgtggactgg	tgtccttcg	gggtttctcc
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	1861	ctccatccgc	atggacaatc	ccttttaccc	acggtggctg	gagaaggaa	caaaggaccc
	1921	tctggtgaag	ctcttcgtgc	gagaacctga	gaagaggctg	ggcgtgagggg	gagacatccg
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2041 accgttccgg ccgaaaagtga aatcaccatt tgactgcagc aatttcgaca aagaattctt  
 2101 aaacgagaag ccccggtctgt catttgcga cagagcactg atcaacagca tggaccagaa  
 2161 tatgttcagg aacttttcct tcataaacc cggatggag cggctgatcat cctgaatctt  
 2221 gcccctccag agacaggaaa gaatttgcct tgcacttggg aactggtca agagacactg  
 5 2281 cttgggttcc ttttcaact tggaaaaaga aagaaacact caacaataaa gactgagacc  
 2341 ctgcggcccc catgtgactt ttatctgttag cagaaaccaa gtctacttca ctaatgacga  
 2401 tgccgtgtgt ctcgtctcct gacatgtctc acagacgctc ctgaagtttag gtcattacta  
 2461 accatagtta ttacttgaa agatgggtct cgcacttgg aaaggttca agacttgata  
 2521 ctgcaataaaa ttatggctct tcacctggc gccaactgct gatcaacgaa atgcttgg  
 10 2581 aatcaggggc aaacggagta cagacgtctc aagactgaaa cggcccccatt gcctggctca  
 2641 gtagcggatc tcactcagcc gcagacaagt aatcaactaac ccgtttattt ctattcctat  
 2701 ctgtggatgg gtaaatgctg gggccagcc ctggataggt tttatggga attc

15

DEFINITION Homo sapiens mRNA for AMP-activated protein kinase beta 2 subunit.

ACCESSION AJ224538

VERSION AJ224538.1 GI:2916801

FEATURES Location/Qualifiers

source 1..819  
 /organism="Homo sapiens"  
 /db\_xref="taxon:9606"

25 gene 1..819  
 /gene="AMPK beta 2"

CDS 1..819  
 /gene="AMPK beta 2"  
 /function="regulatory subunit"  
 /codon\_start=1  
 /evidence=experimental  
 /product="AMP-activated protein kinase beta 2  
 subunit"  
 /protein\_id="CAA12030.1"  
 /db\_xref="GI:2916802"  
 /db\_xref="SWISS-PROT:O43741"

30

35

/translation="MGNNTTSDRVSGERHGAKAARSEGAGGHAPGKEHKIMVGSTDDPS  
 40 VFSLPDSKLPGDKEFVSWQQDLEDSVKPTQQARPTVIRWSEGGKEVFISGSFNNWSTK  
 IPLIKSHNDFVAILDLPEGEHQYKFFVDGQWVHDPEPVVTSQLGTINNLHVKKSDF  
 EVFDALKLDSMESSETSCRDLSSEPPGPYQEMYAFRSEERFKSPPILPPHLLQVILN  
 45 KDTNISCDPALLPEPNHVMLNHLYALSIKDSVMVLSATRYKKYVTTLLYKPI"  
 BASE COUNT 208 a 215 c 197 g 199 t

ORIGIN

50 1 atggaaaaca ccaccagcga cgggtgtcc ggggagcgcc acggcgccaa ggctgcacgc  
 61 tccgagggcg caggcgccca tgccccgggg aaggagcaca agatcatggt ggggagtagc  
 121 gacgacccca gcgtgttcag cttccctgac tccaagctcc ctggggacaa agagtttgta  
 181 tcatggcagc aggatttgg aactccgt aagcccacac agcaggcccg gcccactgtt  
 241 atccgctggt ctgaaggagg caaggaggc ttcatctctg ggtctttcaa caattggagc  
 301 accaagattc cactgattaa gagccataat gactttgtt ccattctggc ctccttgag  
 361 ggagagcacc aatacaagtt ctttgttat ggacagtggg ttcatgatcc atcagagcc  
 421 gtggttacca gtcagcttgg cacaattaac aatttgcattt atgtcaagaa atctgatttt  
 481 gaggtgttcg atgctttaaa gtttagattt atggaaagt ctgagacatc ttgttagagac  
 541 cttccagct caccggcagg gccttatggt caagaaatgt atgcgtttcg atctgaggaa  
 60 601 agattcaaat ccccacccat cttccctctt catctactt aagttatttt taacaaagac  
 661 actaatattt cttgtgaccc agcctactc cctgagccca accatgttat gctgaaccat  
 721 ctctatgcattt tgcattaa ggacagtgtt atggcctta ggcgcaaccat tcgctacaag  
 781 aagaagtatg ttactactt gctataacaag cccatttga

**DEFINITION** Human protein kinase (JNK2) mRNA, complete cds.  
**ACCESSION** U09759  
**VERSION** U09759.1 GI:607785  
**FEATURES**  
 source Location/Qualifiers  
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 /db\_xref="taxon:9606"  
 10 gene 152..1426  
 /gene="JNK2"  
 CDS 152..1426  
 /gene="JNK2"  
 /codon\_start=1  
 /product="protein kinase"  
 /protein\_id="AAA74740.1"  
 /db\_xref="GI:607786"  
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 20 /translation="MSDSKCDSQFYSVQVADSTFTVLKRYQQLKPIGSGAQGIVCAAF  
 DTVLGINVAVKKLSRPFQNQTHAKRAYRELVLLKCVNHKNIISLLNVFTPQKTLEEFQ  
 DVYLVMELMMDANLCQVIHMELDHERMSYLLYQMLCGIKHLHSAGIIHRDLKPSNIVVK  
 25 SDCTLKILDGLARTACTNFMMTPYVVTRYRAPEVILGMGYKENVDIWSVGCIMGEL  
 VKGCVIFQGTDHIDQWNKVIEQLGTPSAEFMKKLOPTVRNYVENRPKYPGIKFEELFP  
 30 DWIFPSESERDKIKTSQARDLLSKMLVIDPDKRISVDEALRHPYITVWYDPAEAEAPP  
 PQIYDAQLEEREHAIEEWKELIYKEVMDWEERSKNGVVKDQPPDAAVSSNATPSQSSS  
 INDISSMSTEQTLASDTDSSLADASTGPLEGCR"  
 35 BASE COUNT 562 a 388 c 427 g 496 t  
 ORIGIN  
 1 caaaactacgt gctgtacagc tgcatcagct gctcgtagac atgtccagca gctggtcgag  
 61 gtccacgccc cggttaggtga agttgcgaa ggccccggcga gggatctgaa acttgcacct  
 121 tacccttcgg gatattgcag gacgtctgcat catgagcgcac agtaaatgtg acagtcatg  
 181 ttatagtgtc caagtggcag actcaacctt cactgtctta aaacgttacc agcagctgaa  
 241 accaatggc tctggggccc aaggaggatgt ttgtctgcata tttgatcacatg ttcttgggat  
 301 aaatgtgtca gtcagaaaaac taagccgtcc ttttcagaac caaactcatg caaagagagc  
 361 ttatcgaaa cttgtctct taaaatgtgt caatcataaa aatataatta gtttggatt  
 421 tttgtttaca ccacaaaaaa ctctagaaga atttcaagat gtgtatgg ttatggatt  
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 841 tattgatcag tggataaaag ttattgagca gctggaaaca ccatcagcag agttcatgaa  
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 961 atttgaagaa ctctttccag attggatatt cccatcagaa tctgacgtc acaaaaataaa  
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 1081 ctctgttagac gaagctctgc gtcacccata catcaactgtt tggatgacc cccggaaagc  
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 1261 tttgttaaaa gatcagcctc cagatgcgc agtaagttag aacggcactt cttctcgt  
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 1561 cagacctgtat aggattgcct gcttagatga taaaatgagg cagaatatgt ctgaaagaaaa  
 1621 aaatttgcac ccacacttct agagattttt ttcagatca tttcgttgc gcaagtttagag  
 1681 taggtqaatt tttcaatttq tactgtqac aqttctcat catctgttaac ttttggatgt

1741 attgtgcatt tgaccacaaa tgcttgcttg gacttgccta tctagcactt tggaaatcag  
 1801 tatttaaatg ccaaataatc ttccaggtag tgctgcttct gaagttatct cttaatcctc  
 1861 ttaagtaatt tgg

5

DEFINITION Human p21-activated protein kinase (Pak2) gene,  
 complete cds.

10   ACCESSION   U24153  
 VERSION       U24153.1   GI:780807  
 FEATURES      source     Location/Qualifiers  
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                 /organism="Homo sapiens"  
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                 /clone="212"  
                 /cell\_type="EBV-transformed peripheral lymphocytes"  
                 /clone\_lib="lambdaYESR human lymphocyte cDNA  
 library;  
 20               gene     Elledge and Spottswood, EMBO 10:2653 (1991) "  
                 40..1617  
                 /gene="Pak2"  
                 40..1617  
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                 /db\_xref="GI:780808"  
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 35               GTEAPAVVTEEDDEETAPPVIAPRPDHTKSIYTRSVIDPVPAVGDSHVGDAAKSL  
                  DKQKKKPMTDEEIMEKLRTIVSIGDPKKKYTRYEKIGQGASGTVFATDVALGQEVA  
 40               IKQINLQKQPKELIINEILVMKELKNPNIVNFLDSYLVGDELFFVUMEYLAGGS LTDV  
                  VTETACMDEAQIAAVCRECLQALEFLHANQVIHRDIKSDNVLLGMEGSVKLTDFGFCA  
                  QITPEQSQRSTMVGT PYWMAPEVVTRKAYGPKVDIWSLGIMAIEMVEGEPPYLNENPL  
 45               RALYLIATNGTPELQNPEKSPIFRDFLNRCLEMDVEKRGSAKELLQHPFLKLAKPLS  
                  SLTPLIMAACEAMKSNR"  
 BASE COUNT     615 a    413 c    481 g    510 t

50   ORIGIN  
 1   gaccttggct tgcccggggc catttcataa ttctgaatca tgtctgataa cggagaactg  
 61   gaagataagg ctccagcacc tcctgtgcga atgagcagca ccatctttag cactggaggc  
 121   aaagaccctt tgtcagccaa tcacagtttgc aaaccttgc cctctgttcc agaagagaaaa  
 181   aagcccaggc ataaaatcat ctccatattc tcaggcacag agaaagggaa taaaaagaaaa  
 241   gaaaaggaac ggccagaaat ttctcctcca tctgattttg agcacaccat ccatgttggc  
 301   tttgatgctg ttactggaga attcactggc atgccagaac agtgggctcg attactacag  
 361   acctccaata tcaccaaact agagcaaaag aagaatcctc aggctgtgt ggatgtccta  
 421   aagtcttacg actccaacac agtgaagcag aaatatctga gctttactcc tcctgagaaaa  
 481   gatggccttc ttctggaaac gccagcactg aatgccaagg gaacagaagc acccgccagta  
 541   gtgacagagg aggaggatga tgatgaagag actgctcctc ccgttattgc cccgcgaccg  
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 661   gattcacatg ttgtatggc tgccaaatgtct ttagacaac agaaaaagaa gcctaagatg  
 721   acagatgaag agattatgga gaaattaaga actatcgtga gcataggtga ccctaagaaaa

781 aaatatacaa gatatgaaaa aattggacaa ggggcttctg gtacagttt cactgctact  
 841 gacgttgcac tgggacagga gggttctatc aaacaaatta attacagaa acagccaaag  
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 1681 aatgatgact ctgttggggg tttaaagaaa tggtctgcat aacctgaatg aaagaaggaa  
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 20 1921 ggtgatttgg tggatagacc catgaatggc ccctgggggt tttcaatctt ggattggagg  
 1981 tgggggttgc agagtgttgc cacgtcttagc tcctctccc

25

DEFINITION Human cyclin-dependent kinase 4 (CDK4) gene,  
complete cds.

ACCESSION U37022

VERSION U37022.1 GI:1353415

30 FEATURES Location/Qualifiers

source 1..4233  
           /organism="Homo sapiens"  
           /db\_xref="taxon:9606"  
           /chromosome="12"  
           /map="12q13"  
           /clone="24C7"

mRNA

join(85..291,729..965,1123..1258,1376..1543,1701..1810,  
       2949..2999,3135..3270,3836..4233)

40 exon /gene="CDK4"  
       /product="cyclin-dependent kinase 4"

exon 85..291

/gene="CDK4"

gene 85..4233

/gene="CDK4"

exon 729..965

/gene="CDK4"

CDS

join(748..965,1123..1258,1376..1543,1701..1810,2949..2999,  
       3135..3270,3836..3928)

50 gene /gene="CDK4"  
       /codon\_start=1  
       /product="cyclin-dependent kinase 4"  
       /protein\_id="AAC50506.1"  
       /db\_xref="GI:1353416"

/translation="MATSRYEPVAEIGVGAYGTVYKARDPHSGHFVALKSVRVPNGGG

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YLDKAPPPGLPAETIKDLMRQFLRGLDFLHANCIVHRDLKPENILVTSGGTVKLADFG

LARIYSYQMALTPVVVTLWYRAPEVLLQSTYATPVDMWSVGCIFAFEMFRRKPLFCGNS

60

EADQLGKIFDLIGLPEDDWPRDVLSPRGAFPPRGPRPVQSVVPEMEESGAQLLLEML  
 TFPNPHKRISAFRALQHSYLNHKDEGNPE"

	BASE COUNT	945 a	1050 c	1101 g	1124 t	13 others	
5	ORIGIN						
1	ccctcctccc	agtcaagca	ccttcgtcc	gcccctcagc	gcattgggtgg	cggtcacgtg	
61	cccagaacgt	ccggcggtcg	ccccgcctc	ccagttccg	cgcccttctt	tggcagctgg	
121	tcacatggtg	agggtggggg	tgagggggcc	tctctagtt	gcccctgtg	tctatggtcg	
181	ggccctctgc	gtccagctgc	tccggaccga	gctcgggtgt	atggggccgt	aggaaccggc	
10	241	tccggggccc	cgataacggg	ccgccccccac	agcaccggg	gctggcgtga	ggtaagtgc
301	gtcccttccc	aggaatgaga	accagtgc	gccccctca	cagtttcca	cgcgttcgtt	
361	tcgcgagctg	gttatggaa	ggtcgctcaa	gggcggaa	ttgggcctt	gtggcatgg	
421	gaaaatataa	tttagggac	tgaggtgtag	gatcttcgt	gcaaggcatg	tgtcatgtgt	
15	481	gatcttgtg	cggggcgcga	ttgtcccaa	ggaaaaagcg	ttttctattg	cagggcctca
541	cgtggctgga	gggggttggt	ttgagtatt	gtgttatctc	ttggggccggc	cccaaggaag	
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661	ttgttgctgc	aggtcatacc	atccatactc	tgtaaagcgac	ttttgggtat	aggagtctgt	
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901	agcacatgc	gtgaggtggc	ttttaggtagg	cgactggagg	ctttagagca	tcccaatgtt	
961	gtccgggtgag	aaagggtggg	agggtgggc	gtggggagta	aaggggaaaag	acagccata	
1021	ggtgggtgt	gatgatctgt	agagaagtgg	ggacccttag	gaaataatga	gaggccatgt	
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1861	tcataaaacca	catccatacc	ttggccattc	ttttttttt	agaccagggc	ttgctgtgtt	
1921	gcccaggctg	gattgcaatg	gcatgatcac	agctcaactgc	agttcaacc	tcctggctc	
1981	aagtgtatcc	cccatctcag	ctttccaaact	agtgacact	acaggcacgc	acctccatgc	
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DEFINITION Human MEK5 mRNA, complete cds.

ACCESSION U25265

VERSION U25265.1 GI:1255719

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25 DEFINITION Homo sapiens a disintegrin and metalloproteinase  
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DEFINITION Homo sapiens a disintegrin and metalloproteinase domain 17  
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 variant 1, mRNA.  
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 1561 cccagagtg ttttccagaa cgcagcaata aagtttgg gaaactcgagg gtggatgaa  
 1621 gagaagagtg tgatcctggc atcatgtatc tgaacaacga cacctgctgc aacagcgact  
 1681 gcacgttggaa ggaagggttc cagtcagtg acaggaacag tccttgcgt aaaaactgtc  
 1741 agttttagac tgcccagaag aagtgcaggc aggcattaa tgctacttgc aaggcgtgt  
 1801 cctactgcac aggttaatagc agtgagtgcg cgcctccagg aatgctgaa aatgacactg  
 1861 tttgcttggc tcttggcaag tgtaaggatg gaaaatgc cctttctgc gagagggaa  
 1921 agcagcttggc gtcctgtgca tgtaatgaaa ctgacaactc ctgcaagggt tgctgcagg  
 1981 acctttctgg cccgtgtgt ccctatgtcg atgctgaaca aaaaacttta tttttgagga  
 2041 aaggaaagcc ctgtacagta ggattttgtg acatgaatgg caaatgttag aacagactac  
 2101 aggatgtat tgaacgattt tggatttca ttgaccagct gacatcaat acttttggaa  
 2161 agtttttagc agacaacatc gttgggtctg tcctgggtt ctccttgcata ttttgattc  
 2221 ctttcagcat tcttgcatt tggtggata agaaatggtaaaacagtat gaatctctgt  
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 2341 ttatcaaacc ctttcctgca ccccaacttgc caggccgcct gcaagcctgcc cctgtatcc  
 2401 ctgcggcc accagctcca aactggacc accagagaat ggacaccatc caggaagacc  
 2461 ccagcacaga ctccccatag gacgaggatg gtttggagaa ggaccccttc ccaaatacgca  
 2521 gcacactgc caagtcttgc gaggatctca cggaccatcc ggtccggaga agtggaaaagg  
 2581 ctgccttgc taaacttgc gtcgaaatgc gtgttaacag caaagaaaca gagtgcataat  
 2641 ttatgttca gtccttgc cttaagtgtg caaaaatattt ttatgattt gacccatcaaaa  
 2701 tcaatcacag ttgttattttt gtaagactg ggaagtact tagcagatgc tggtcatgtg  
 2761 tttgaatctc ctgcaggtaa acatgttgc tttgggttgg cccttctctt tttgaaaagg  
 2821 taaggtaaaa gtaatctac ttatgttgc gcttccagg tttatgtttt aaaaatctt  
 2881 ttgactgtg gtcggatgg gttatgataa tttacgtttt

2941 tgtaaaattaa tcctttatata tgataaacgc actgactagg gaaatgatca gtttttttt  
3001 atacactgta atgaaccgct gaatatgaag catttggcat ttatttgtga gaaaagtgga  
3061 atagttttt tttttttttt ttttttttgc cttcaactaa aaacaaagga gataaattta  
3121 gtatacattg tatctaaattt gtgggtctat ttctagttat tacccagagt ttttatgttag  
5 3181 caggaaaaat atatatctaa atttagaaat catttgggtt aatatggctc ttcataattc  
3241 taagactaat gctcagaacc taaccactac cttacagtga gggctataca tggtagccag  
3301 ttgaatttt ggaatctacc aactgtttag ggccctgatt tgctggcag ttttctgtta  
3361 ttttataagt atcttcatgt atccctgtta ctgataggga tacatgtctt agaaaattca  
3421 ctattggctg ggagtggctt ctcatgcctg taatcccagc acttggagag gctgaggttg  
10 3481 cgccactaca ctccagcctg ggtgacagag tgagatctgc ctc

Accession number: NM\_007026

Definition: DUSP14, MKP-L

15 GTGGCCCGCAGGAGGACGGAGGCCCTAACCGCAACCCGCCGCCGCCGCCGATTTGATTGTATCCAC  
TGTCACCAGCACTGCTCACTTAGGACTTTCTGGATCCAGACCCAGGCAGCGCACACTGGACTCTTGAGGA  
AGAAGGAGACTCTAATTTGGATTCTTGGTGGAGGAAAATAAAACACTCTGGTCTGCCGCCAACGATG  
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TCGGATGATTCCGAGGGAGACATAGGAGGCATTGCTCAAATCACCTCTCTATTCTGGCAGAGGC  
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TCATTAACCCCTTAGAGACAAGCTTGCCCCAGGCTGCGGACCAGACAGATGCTTAGGAAAGGTTGATAC  
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AGTTAAATACACCCATTATTTAGCTGTTAAGTAACATATAATGAAATCTGCTGCAAAATCTCTGGAA  
35 ATCCATGTGCCAGGATTATATTAGCATTATTTAATAAAATCTATATGCTAAAAAAAAAAAAAA  
A

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization International Bureau



(43) International Publication Date  
3 July 2003 (03.07.2003)

PCT

(10) International Publication Number  
WO 2003/054228 A3

(51) International Patent Classification<sup>7</sup>: C12Q 1/42, 1/48, 1/37, 1/68, C07K 16/00, A61K 39/42

(74) Agents: GINZEL, Christian et al.; c/o Zimmermann & Partner, Postfach 330 920, 80069 Munich (DE).

(21) International Application Number:  
PCT/EP2002/014578

(22) International Filing Date:  
19 December 2002 (19.12.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:  
60/341,757 21 December 2001 (21.12.2001) US

(71) Applicant (for all designated States except US): AXXIMA PHARMACEUTICALS AG [DE/DE]; Max-Lebsche-Platz 32, 81377 München (DE).

(72) Inventors; and

(75) Inventors/Applicants (for US only): SALASSIDIS, Konstadinos [GR/DE]; Hauptstrasse 14c, 85386 Eching (DE). SCHUBART, Daniel [DE/DE]; Avenue Charles de Gaulle 1c, 13469 Berlin (DE). GUTBROD, Heidrun [DE/DE]; Weberhofstrasse 8, 82166 Graefelfing (DE). MUELLER, Stefan [DE/DE]; Thalkirchnerstrasse 184, 81371 Munich (DE). KRAETZER, Friedrich [DE/DE]; Geisenbrunnerstrasse 51, 81475 Munich (DE). OBERT, Sabine [DE/DE]; Bellinzonastrasse 17, 81475 Munich (DE).

(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— with international search report

(88) Date of publication of the international search report:  
15 January 2004

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

A3

(54) Title: HUMAN CELLULAR ENZYME AS TARGETS AGAINST HEPATITIS C VIRUS INFECTIONS

(57) Abstract: The present invention relates to human cellular protein kinases, metalloproteases and one phosphatase: beta-adrenergic receptor kinase 1 (NM 001619), Mitogen activated protein kinase activated protein kinase 5 (AF032437), Insulin-stimulated protein kinase 1 (U08316), Discoidin domain receptor family, member 1(NM 013994), Protein Kinase C, mu (X75756), Protein Kinase C, theta (L01087), AMP-activated protein kinase beta 2 subunit (AJ 224538), JNK2 (U09759), Human p21-activated protein kinase 2 (U24153), cyclin-dependent kinase 4 (U37022), MEKS (U25265), MKP-L (NM 007026), ADAM22 (NM 016351), and ADAM17 (U92649) as potential targets for medical intervention against Hepatitis C virus (HCV) infections. The present invention relates also to a method for the detection of compounds useful for prophylaxis and/or treatment of Hepatitis C virus infections, a method for detecting Hepatitis C virus infections in an individual or in cells. Mono- or polyclonal antibodies are disclosed effective for the treatment of HCV infections together with methods for treating Hepatitis C virus infections or for the regulation of Hepatitis C virus production and/or replication wherein said antibodies may be used. Finally the present invention relates to a solid support useful for detecting Hepatitis C virus infections or for screening compounds useful for prophylaxis and/or treatment of HCV infections.

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 02/14578

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7	C12Q1/42	C12Q1/48	C12Q1/37	C12Q1/68	C07K16/00
	A61K39/42				

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 C12Q C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, EPO-Internal, EMBASE, MEDLINE, BIOSIS

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BRANCH ANDREA D ET AL: "The coming impact of gene expression profiling on the diagnosis and treatment of HCV-associated liver disease." ANTIVIRAL RESEARCH, vol. 52, no. 2, November 2001 (2001-11), pages 173-179, XP002249604 ISSN: 0166-3542 the whole document ----	1-14
X	BIGGER CATHERINE B ET AL: "DNA microarray analysis of chimpanzee liver during acute resolving hepatitis C virus infection." JOURNAL OF VIROLOGY, vol. 75, no. 15, August 2001 (2001-08), pages 7059-7066, XP002249605 ISSN: 0022-538X the whole document ----	1-14
	-/-	

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the International search

30 July 2003

Date of mailing of the International search report

21/08/2003

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Niemann, F

## INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 02/14578

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>YAMASHITA TARO ET AL: "Serial analysis of gene expression in chronic hepatitis C and hepatocellular carcinoma."          BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS,          vol. 282, no. 2,          30 March 2001 (2001-03-30), pages 647-654,          XP002249606          ISSN: 0006-291X          the whole document</p> <p>---</p>	1-14
X	<p>HUANG YING ET AL: "A human hepatoma cell line expressing hepatitis C virus nonstructural proteins tightly regulated by tetracycline."          BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS,          vol. 281, no. 3,          2 March 2001 (2001-03-02), pages 732-740,          XP002249607          ISSN: 0006-291X          the whole document</p> <p>-----</p>	1-14

# INTERNATIONAL SEARCH REPORT

...nternational application No.  
PCT/EP 02/14578

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1.  Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:  
**see FURTHER INFORMATION sheet PCT/ISA/210**
2.  Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:  
**see FURTHER INFORMATION sheet PCT/ISA/210**
3.  Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

**see additional sheet**

1.  As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3.  As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4.  No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

The additional search fees were accompanied by the applicant's protest.  
 No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

## Continuation of Box I.1

Although claims 2, 3, are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Although claims 6-9 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition

## Continuation of Box I.2

Present claims 6-7, 14 relate to an agent defined by reference to a desirable characteristic or property, namely inhibiting at least partially the activity of at least one of the enzymes cited in claim 1.

The claims cover all agents having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such agent. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the agent by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to antibody which binds to at least one of the enzymes cited in claim 1 (see claim 8).

Present claims 10-11 relate to an oligonucleotide defined by reference to a desirable characteristic or property, namely being capable of detecting activity of at least one of the enzymes cited in claim 1.

The claims cover all oligonucleotides having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such oligonucleotide. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the oligonucleotide by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to oligonucleotides encoding one of the enzymes cited in claim 1 (see claim 12).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1 : 1-14 (all partially)

method for detecting compounds useful for the prophylaxis and/or treatment of Hepatitis C virus infection, the method comprising contacting a test compound with at least beta-adrenergic receptor kinase 1  
method for detecting Hepatitis C virus infection

Inventions 2-14 : 1-14 (all partially)

method for detecting compounds useful for the prophylaxis and/or treatment of Hepatitis C virus infection, the method comprising contacting a test compound with at least one of the enzymes mentioned in claim 1  
method for detecting Hepatitis C virus infection

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